



SEDIMENT TOXICITY TEST REPORT

FOR

BIO SOLUTIONS, LLC

BIO CLEAN 2 – 10% V:V

REQUESTED BY: BIO SOLUTIONS, LLC
SKIP BOURDIER
2515 CUMMINS ROAD
HOUMA, LA 70360

PREPARED BY: 
DIANNE BOURQUE
ADMINISTRATIVE ASSISTANT

3/18/20
DATE

APPROVED BY: 
JON RICHARDSON
TECHNICAL MANAGER, BIOASSAY SERVICES

3/18/20
DATE

THE RESULTS OF THIS ANALYSIS RELATE ONLY TO THE REFERENCED SAMPLE AS IT WAS SUBMITTED TO
ELEMENT MATERIALS TECHNOLOGY, LLC.

STATE OF LOUISIANA LABORATORY ACCREDITATION CERTIFICATE NO. 01997

Job Number: 20020750

1. SAMPLE INFORMATION

Product Name: Bio Clean 2
Name of Sampler/Company Skip Bourdier
Date of Sample: 02.18.20
Time of Sample: 1115
Type of Sample: Added to Field SBM at 10% V:V
Dates of Sediment Toxicity Test: 02.20.20 to 02.24.20

ANALYTICAL METHOD USED:

This test method conforms to the method in NPDES Permit GMG 290000-Appendix A, published October 1, 2012.

2. TEST SUMMARY

LC₅₀ Value for the Reference Drilling Fluid + Product: 32.6 ml/Kg

Associated 95% Confidence Interval: 29.8 to 35.6 ml/Kg

LC₅₀ Value for the Submitted SBM sample: 96.5 ml/Kg

Associated 95% Confidence Interval: 81.3 to 116 ml/Kg

Results: $\frac{32.6 \text{ ml/Kg}}{96.5 \text{ ml/Kg}} = 0.3 = \text{Sediment Toxicity Ratio}$

PASS/FAIL Statement: Since the above STR (Sediment Toxicity Ratio) of **0.3 is not greater than the limit of 1.0** as specified in the modification of the NPDES Permit GMG 290000, Appendix A, for Gulf of Mexico Discharges, this sample would **PASS** the required testing parameter.

Statistical Method used: Probit & Trimmed Spearman-Kärber

Leptocheirus plumulosus, that passed through a 1000µm mesh sieve but were collected on 850µm and 710µm mesh sieve, were used in a 50/50 combination. These animals were then exposed to varying concentrations of the submitted Synthetic Based Drilling Fluid sample and formulated sediment. At the same time, they were also exposed to the appropriate Reference Drilling Fluid and the same sediment. At the end of the 96-hour tests, LC₅₀ values were calculated utilizing CETIS v1.8.7.12.

3. MATERIALS AND METHODS

The materials utilized and the methods employed in this test were based on those suggested by the U. S. Environmental Protection Agency in the October 1, 2012 re-issuance of NPDES GMG 290000-Appendix A and the API Sediment Toxicity Work Group's Unified Method SOP

a. **Artificial Seawater Preparation**

Artificial seawater was prepared by mixing distilled water with synthetic sea salts (Crystal Seas Marine Mix). This water was made to a salinity of 20 ± 1 ppt.

b. **Organism Acquisition and Maintenance**

L. plumulosus were obtained from in-house cultures maintained by Element Materials Technology Lafayette, LLC. In order to ascertain the health and strength of the test animals, a 96-hour reference toxicant test was conducted using Cadmium chloride (Fisher Scientific, Lot No. 142892) as the toxic material. The resultant LC_{50} for the test beginning on 02.20.20 was 2.1 ppm.

c. **Synthetic Based Drilling Fluid Characteristics and Storage**

Mud weight: N/A (Reference Fluid used was 14.5 ppg NDFH 01.27.20)

Whole mud Temperature: 1.7°C upon receipt into the lab. Physical condition: good, no foul odors or oily spots on container walls

Sample storage: 4°C , in dark, under blanket of Nitrogen

d. **Sediment Characteristics**

Sediment used: Formulated – FS 17.1

Moisture content: 49.4%

e. **Mixing Procedure:**

The appropriate amounts of SBM sample and sediment are weighed into stainless steel mixing bowls. They are then mixed for 10 minutes with a Kitchen Aid Model KHM6 hand-held mixer equipped with stainless steel blades. The control sediment is mixed in the same manner. The homogeneity of these mixtures should have a coefficient of variation of less than or equal to 20%. This is attainable by following proper mixing procedures. This CV will be checked on the highest and lowest of the concentrations at least once per year. Proper CVs are being obtained at this time.

4. EXPERIMENTAL CONDITIONS

The 96 hour sediment toxicity test was conducted at a salinity of 20 ± 1 ppt and a temperature of $20 \pm 1^{\circ}\text{C}$. Dissolved oxygen, temperature, salinity and pH were measured at 0, 24, 48, 72, and 96 hours. Ammonia is also monitored in the overlying water of the test to make sure it stays within the tolerance range of the *L. plumulosus*. The test bottles were aerated throughout the test period. The test was aerated with the air-flow delivered via TYGON Microbore tubing at a rate between 50 – 140 cc/min. Illumination was provided at an intensity of 500 – 1000 lux using cool white fluorescent bulbs in a cycle of 14 hours light and 10 hours dark. The temperature in the test chamber was monitored and recorded.

5. EXPERIMENTAL PROCEDURES

For each test concentration, 150 ml of the drilling fluid/sediment mixture is added to a 1L glass jar. 600 ml of overlying saltwater is then added. Each test is performed using 5 concentrations and a control. Each concentration and the control have 5 replicates. The sediment mixture and water are added to the test vessels a day before the organisms are placed into the vessels. This allows for settling of any suspended particles. When the *L. plumulosus* are randomized for the test, they are counted out individually until a medicine cup contains 10 organisms. Two medicine cups with leptos are then combined into one, and that cup, containing 20 leptos is added to a randomly placed test vessel. The test vessel is then covered and aeration adjusted. The test water is not renewed and the organisms are not fed during the 96-hour test period.

6. TEST ACCEPTABILITY

A 96-Hour SBM toxicity test shall be deemed unacceptable if the control has a survival rate of less than 90%, or if the Coefficient of Variation (CV) for the Control's survival is not less than 40%. For this test, the Survival Rate for the Test sample's Control was 98% and the CV for the Control was 2.79%. The Reference test's Control has a Survival rate of 100% with a CV of 0.0%.

7. RESULTS

- a. Method of analysis: Probit & Trimmed Spearman-Kärber
- b. Results: The ratio of the SBM LC₅₀ vs. Reference Drilling Fluid LC₅₀ is **0.3**
- c. **PASS/FAIL Statement:** Since the above STR (Sediment Toxicity Ratio) of **0.3 is not greater than the limit of 1.0** as specified in the modification of the NPDES Permit GMG 290000, Appendix A, for Gulf of Mexico Discharges, this sample would **PASS** the required testing parameter.
- d. Testing Facility: Element Materials Technology Lafayette, LLC
Bioassay Division
2417 West Pinhook Road
Lafayette, LA 70508

8. REFERENCES

U. S. Environmental Protection Agency, 2011 Analytical Methods for the Oil and Gas. Extraction Point Source Category. Method 1644.

U. S. Environmental Protection Agency, October 1, 2012. Notice of Final NPDES General Permit. GMG 290000 – Appendix A

API Sediment Toxicity Work Group – Unified Method SOP